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The Journal of Infectious Diseases

FOUNDED BY THE MEMORIAL INSTITUTE FOR INFECTIOUS DISEASES

Vol. 5

March 30, 1908

No. 2

THE FREQUENCY OF TUBERCLE BACILLI IN THE MARKET MILK OF THE CITY OF WASHINGTON, D. C.*

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INTRODUCTION.

Numerous investigators in recent years have shown the infectiousness for animals of milk containing tubercle bacilli. Whether the milk from animals with tuberculosis but with healthy udders contains tubercle bacilli is not definitely settled. Many prominent scientists seem to have known that at times the milk from such animals does contain tubercle bacilli virulent for laboratory animals, but in view of recent work there may be some doubt as to whether the bacilli really passed through the udder or gained access to the milk from contamination with feces containing tubercle bacilli.

Schroeder and Cotton¹ have recently shown that cows so slightly affected with tuberculosis as only to be discoverable by the tuberculin reaction pass virulent bacilli in their feces. Many believe that milk from a tuberculous cow with unaffected udder is free from infection

^{*} Received for publication February 4, 1908.

¹ Bull. No. 99, Bureau of Animal Industry, 1907.

and becomes infected from the feces of the animal or from its environment. This observation is of the very greatest importance, and if confirmed shows more than ever that the greatest care is necessary in guarding milk from contamination from the time it is drawn until it is consumed.

The milk supply of many of the cities of Europe and England has been examined for tubercle bacilli. Most observers have used the animal test, i. e., they have injected various amounts into guinea-pigs or rabbits. The percentage of samples showing tubercle bacilli has varied between very wide limits, no doubt dependent upon the difference in the number of tuberculous cows in the herds supplying milk to the different cities and on differences in technic. Some observers have found that when a number of animals are inoculated with the same samples of milk only one, perhaps, will develop tuberculosis. Some centrifugalized the milk and gave sediment alone, while others gave sediment and cream.

I will not enter into the question as to whether the tubercle bacilli found in milk are virulent for man, but give my results only as determining whether the market milk of the city of Washington contains tubercle bacilli virulent for guinea-pigs.

THE NUMBER OF TUBERCULOUS COWS IN THE DAIRIES SUPPLYING WASHINGTON, D. C.

A letter was addressed to Dr. W. C. Woodward, health officer, Washington, D. C., and to the Agricultural Department requesting data as to the number of cows in dairies supplying milk to the city of Washington, which had responded to the tuberculin test. Dr. J. R. Mohler stated in October, 1907, that of 1,147 recently tested cows supplying milk to the city of Washington, 214 or 18.6 per cent, responded to the tuberculin test. He stated that he did not consider this a fair estimate of the extent of tuberculosis in the dairy herds of this vicinity as the tests were being applied only to those herds which had recently been cleansed by private tests or appeared so healthy that their owners had no fear of having them tested.

I am informed by the District Health Department that 1,059 cows from 51 herds in Virginia, Maryland, and the District of Columbia supplying milk to the city of Washington were tested for their reaction

to tuberculin; of this number 160, or 15.1 per cent of the total number of cows tested, responded to the tuberculin test.

Of course the figures above furnished by the Department of Agriculture and the District Health Department do not give a fair idea of the prevalence of tuberculosis in the herds supplying milk to Washington, as only the owners of those herds who had reason to think that their cows were free from tuberculosis permitted the test to be made. If the test had been applied to all the cows supplying milk to the District I have no doubt that the percentage would be very much higher than the figures above would seem to indicate.

The following figures by Salmon¹ show the number and percentage of cattle carcasses condemned for tuberculosis during the years 1901-5 in the meat-inspection service of the Bureau of Animal Industry:

TABLE 1.

Year	Number Carcasses Examined	Percentage of Carcasses Condemned		
1901	5,219,149	0.10		
1902	5,559,969	0.14		
1903	6,134,410	0.14		
1904	6,350,011	0.16		
1905	6,096,597	0.18		

This does not show the total number of animals affected with tuberculosis, for in many cases only a part of the carcass was condemned, and probably many had the disease so slightly that the entire carcass was passed as fit for food. The following table, also taken from Salmon's article showing the results of the tuberculin test of cattle in some states, is of value in showing the wide distribution of cattle tuberculosis. It must be remembered that most of the herds tested were suspected herds which may account for the very high percentages found. (Table 2.)

The results of some of the earlier workers are open to criticism in view of Rabinowitch's discovery of an acid-fast bacillus in butter morphologically similar to the tubercle bacillus. If guinea-pigs are inoculated with milk or butter containing the acid-fast butter bacillus they may often die, and will present lesions to the naked eye very similar to those produced by the tubercle bacillus.

Bull. No. 38, Bureau of Animal Industry, 1906.

TABLE 2.									
RESULTS OF THE	TUBERCULIN	TESTS	OF	CATTLE	IN	VARIOUS	States.		

State	Number Tested	Number Tuberculous	Percentage Tuberculous
Vermont	60,000	2,390	3.9
Massachusetts	24,685	12,443	50.0
Massachusetts, entire herds	4,003	1,080	26.4
Connecticut	6,300		14.2
New York, 1894	947	66	6.9
New York, 1897-98	1,200	163	18.4
Pennsylvania	34,000	4,800	14.1
New Jersey	2,500		21.4
Illinois, 1897-98	020		12.0
Illinois, 1899	3,655	560	15.3
Michigan			13.0
Minnesota	3,430	1	11.1
Iowa	873	122	13.8
Wisconsin:			"
Experiment station tests:			
Suspected herds	323	115	35.6
Non-suspected herds	935	84	0.0
State veterinarian's tests:	300		
Suspected herds	588	101	32.5
Tests of local veterinarians under state veterinarian on	3	-,-	
cattle intended for shipment to states requiring tu-			
berculin certificate	3:421	76	2.2

COLLECTION OF SAMPLES AND TECHNIC.

The samples of milk were all collected and brought to the Hygienic Laboratory by an inspector of the Health Department of the District of Columbia. Usually a pint bottle, though sometimes a quart, with the paper cap untampered with was obtained either from the dairy or delivery wagon; the bottle was at once placed on ice by the collector and usually reached the laboratory in about one hour after collection. A few samples were obtained from some of the hospitals and charitable institutions of the District. The milk and cream were well mixed by vigorously shaking the bottle. The sample for plating was taken out with a sterile pipette and then 50 c.c. of the mixed milk was put into a large sterile centrifuge flask; to the 50 c.c. of milk 100 c.c. of sterile water was added; the flask was then put into the centrifuge machine and centrifugated for one hour at about 2,000 revolutions per minute. The milk was diluted with twice its volume of water with the idea that it would decrease the specific gravity of the milk and so permit of the easier sedimentation of the tubercle bacilli. Usually only one animal was inoculated from each sample, though in some cases two animals were used. Guinea-pigs, largely those raised in the laboratory, of as uniform weight as obtainable, were inoculated with 5 c.c. of the sediment of this centrifugalized mixture of milk and water; the inoculation was made subcutaneously in the belly wall. For each

guinea-pig a different syringe was used. All of the guinea-pigs (usually eight, the number of daily samples) inoculated on the same day were kept in the same cage; those that remained healthy being controls on the environment, etc. The guinea-pigs were examined for enlarged glands after about four weeks, and those with enlarged glands were separated from the others so as to avoid the danger of infecting their companions if the glands broke down.

Many of the animals inoculated died from acute infections with the millions of other bacteria in the milk. Autopsies were made of all the animals that died, but no attempt was made to determine the causal organisms other than the tubercle bacillus.

Those guinea pigs that did not die in at least two months were chloroformed after having been tested by tuberculin, and careful autopsies were made on each animal. Smears, cultures, and sections were made from the various organs of the animals that showed any change from the normal. The smears were stained with carbol-fuchsin and examined for acid-fast bacilli. Cultures were made on glycerinized potato and glycerin-agar; in no instance did any of the cultures show a quick-growing acid-fast organism resembling in any way Rabinowitch's butter bacillus. The sections were stained with carbol-fuchsin for tubercle bacilli and also with hemalum and eosine for histological appearances. The above details were carried out with few exceptions in all of the animals that gave a positive result.

It occurred to me that those animals having tuberculosis might be differentiated from those having other infections by giving all of the guinea-pigs alive at the end of two months a sufficient dose of tuberculin to cause the death of the tuberculous animal in less than 24 hours. Several preliminary tests on known tubercular animals showed that 2 c.c. of crude tuberculin given subcutaneously would almost invariably cause the death of such a guinea-pig in from 6 to 18 hours. As high as 7 c.c. of the same tuberculin given to a healthy pig caused only a temporary discomfort passing off in a few hours. A rather hasty search of the literature failed to show that this idea of giving an amount of tuberculin sufficient to cause the death of a tubercular animal as a means of differentiating true tuberculosis from infection with other acid-fast organisms had ever been used by previous workers. The febrile reaction in a sick guinea-pig on account of

the great variation in the temperature of the animal from handling, etc., is too variable a factor, and a more definite reaction, such as the death of the animal, is necessary. The technic was as follows: All of the animals, in lots of about 30, were given early in the morning

T	A	В	т	T	_
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Dairy	No. of Samples	No. of Samples Lost by Acute Death of Guinea-Pig	No. of Samples Re- maining	No. of Samples Positive for Tuberculosis	Dairy	No. of Samples	No. of Samples Lost by Acute Death of Guinea-Pig	No. of Samples Re- maining	No of Samples Positive for Tuberculosis
1	3	2	I	0	Brought For'd	144	24	120	6
3	3 1	0	3 1	0	55	3 2	I	2 2	0
4	2	0	2	0	57	1	0	1	0
5	2	1	I	0	58	2	0	2	0
6	3 1	0	3 1	0	59	I	0	I	0
8	2	0	2	0	61	2	ő	2	ō
9	4	1	3	0	62	I	0	1	1
10	2 8	0 2	6	0	63	1 2	0	1 2	0
II	1	1	0	0	65	2	I.	1	
13	ī	0	1	0	66	2	0	2	0
14	I	0	1	0	67	1	0	I	0
15	2 I	0	I I	0	68	3 4	I	3	0
17	ī	o	ī	ō	70	3	ī	2	٥
18	3	0	3	1	71	1	0	I	0
19	4	I 0	3 5	0	72	2 5	0	2 4	0 2
21	5	1	4	0	74	3	ī	2	ō
22	3	1	2	0	75	2	0	2	0
23	1	0	1 4	0	76	2 2	0	2 I	0
24 25	5 3	0	3	0	78	ī	0	ī	o
26	ĭ	0	1	0	79	1	0	r	0
27	4	1	3	0	80 81	6	0	2 5	0
28	4 3	0	3	0	82	7	2	5	0
30	2	0	2	0	83	I	0	5 1	٥
31	I	0	1	0	84	6	0	5 2	3
32	3 4	1 2	2 2	0	85 86	2 3	0	3	1
34	3	ō	3	0	87	2	0	2	0
35	2	0	2	0	88	2	0	2 1	0
36	I I	0	I I	0	89	I I	0	0	0
37······ 38·····	4	I	3	ŏ	91	2	ō	2	0
39	4	1	3	0	92	3	1	2	0
40	2	0	2	0	93	3 1	0	3 1	0
41	5 1	0	4 1	0	95	8	3	5	C
43	3	0	3	0	96	3	0	3	0
44	5	0	5	0	97	2	0	2	0
45	4 2	1 0	3 2	2 I	98	4 3	I 2	3	0
46 47	2	ī	1	ō	100	3	î	2	0
48	1	0	1	0	101	1	0	r	0
49	3	0	3	0	102	4	I	3	· O
50	2 2	0	2 2	0	103	4 4	2	2	o
52	2	I	r	0					
53	4 2	I 0	3 2	0	Totals	272	49	223	15
54							18%	82%	6.72%
Carried For'd	144	24	120	6					
							<u> </u>		

2 c.c. of the tuberculin subcutaneously; they were closely watched, and as soon as an animal appeared sick it was placed aside; as soon after death as possible the animal was autopsied; smears, cultures, and sections were made. Of all the guinea-pigs (about 250) that received the tuberculin, no animal died that did not have tuberculosis. Two or three that had slight lesions did not die, but became sick. It was noted that all of the animals died whose lesions had caseated. The reaction, I think, was of distinct service in eliminating infections with other acid-fast organisms. The suggestion is made that with some modification the procedure may have a distinct place as an aid in differentiating true tuberculosis from infections with other acid-fast organisms which produce tubercular-like lesions.

Samples of milk were examined from 104 different dairies; 10 samples from seven hospitals and asylums are also included in this list, being charged also to the dairy supplying the milk.

It is interesting to note that where two guinea-pigs were inoculated with the same samples of milk, in two instances both animals showed tuberculosis, and in two instances only one was positive.

Table 3 gives a summary of the protocols. It shows the laboratory number of the dairy, number of samples from each dairy, number of samples lost by the animal dying in less than three weeks of other infections, number of samples remaining for observation, and total number of samples for each dairy positive for tuberculosis.

The same details are shown in Table 4 for the milk collected from the charitable institutions.

Hospital	Number of Samples	Number of Samples Lost by Acute Death of Guinea-Pig	Number of Samples Remaining	Number of Samples Posi- tive for Tuber- culosis	Remarks
Providence	2 I I 2 I I	0 0 0 0	2 1 1 2 1 1	0 0 0 0 0 1 (a)	(a) Both pigs from sample
Columbia	2	ı	I	0	positive
Totals	10	I	9	I	
		10%	90%	11.1%	

TABLE 4.

SUMMARY.

It will be seen from the tables that of 272 samples of milk, 49 or 18 per cent of the samples were lost by the animal dying in less than three weeks and before sufficient time had elapsed for it to develop tuberculosis. Attention is invited to the fact that the milk from some of the dairies killed acutely a high percentage of all of the animals to which it was given.

Of the 272 samples 223 or 82 per cent remained for study.

Of the 223 that remained, 15 or 6.72 per cent contained sufficient tubercle bacilli to cause typical tuberculosis in the inoculated animals.

Of the samples of milk from 104 dairies two were lost by acute death of the animals, leaving 102; the milk from 11 of these 102 dairies contained tubercle bacilli; this gives a percentage of 10.7 of the dairies examined showing tubercle bacilli in the milk supplied to their customers.

Ten samples of milk were obtained from seven charitable institutions of the District; of these ten samples one was lost by the acute death of the animal, leaving nine samples from six institutions for study. The sample from one institution caused tuberculosis in both guinea-pigs in which it was inoculated.

These results, showing that approximately 11 per cent of the dairies whose milk was examined contained tubercle bacilli virulent for guinea-pigs, do not, however, give a fair idea of the frequency of tubercle bacilli in the market milk of the city of Washington. Attention has already been called to the fact that when two animals were inoculated with the same sample both did not always develop tuberculosis; this might indicate that the bacilli are so few in the amount inoculated that one of the animals by being a little more resistant was able to overcome the infection. The amount inoculated, less than 2 c.c. of milk, is a very small portion of a pint bottle. The creamy layer was not inoculated, and other workers have shown that tubercle bacilli are more frequent in this than in the bottom milk; it is very probable that if more animals had been inoculated with the same sample and both cream and sediment had been used, the percentage of positive results would have been very much higher. The results, however, as they were found, are sufficiently high to emphasize the great necessity for the enactment and rigorous enforcement of a law

requiring that all cows supplying milk to the District be tuberculin tested and free from tuberculosis. This test, which is now universally recognized as a means of determining whether or not an animal has tuberculosis, should be made by a competent veterinarian and those animals that respond should be disposed of in some way so that their milk may no longer be a source of danger to the community.

Note.—For a review of the literature on tubercle bacilli in market milk and for details of the autopsies of my work, see article in *Bull. 41*, *Hygienic Laboratory*, *Public Health and Marine-Hospital Service*, "Milk and Its Relation to the Public Health," by various authors.